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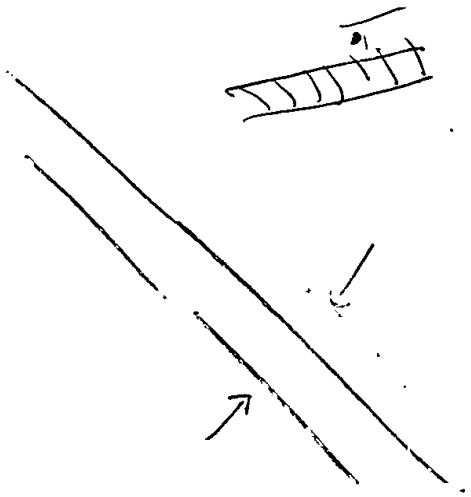
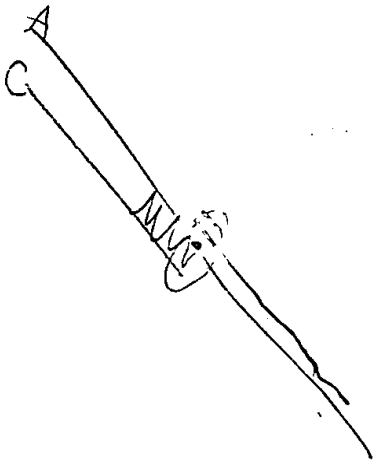
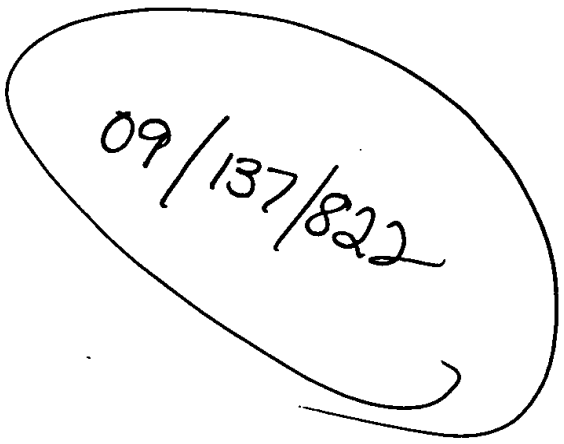
     (FILE 'USPAT' ENTERED AT 12:02:32 ON 20 JUL 1999)
L1        53068 S (TRIPLE OR TRIPLEX OR THREE) (S) (STRAND? OR HELIX)
L2        12650 S (TRIPLE OR TRIPLEX OR THREE) (P) (STRAND? OR HELIX)
L3         5018 S (PNA OR PEPTIDE NUCLEIC ACID)
L4         1012 S L2 AND L3
L5         1012 S L4 AND (DETEC? OR ISOLA? OR PURI?)
L6          967 S OLIGO? AND L5
L7           22 S (TRIPLE OR TRIPLEX OR THREE) (P) (STRAND? OR HELIX)/TI
L8         4259 S (TRIPLE OR TRIPLEX OR THREE) (P) (STRAND? OR HELIX) AND
(DN
L9           13 S ((TRIPLE OR TRIPLEX OR THREE) (P) (STRAND? OR HELIX))/TI
AND (DNA or nucleic acid)
```







```
=> s triple helix or triplex

         32367 TRIPLE
         21376 HELIX
           933 TRIPLE HELIX
                 (TRIPLE(W)HELIX)
          1527 TRIPLEX
L1        2073 TRIPLE HELIX OR TRIPLEX

=> s l1 and quantitat?

         80067 QUANTITAT?
L2         724 L1 AND QUANTITAT?

=> s hybridiz?

L3       17694 HYBRIDIZ?

=> s l2 and l3

L4         617 L2 AND L3

=> s l194a)l2

UNMATCHED RIGHT PARENTHESIS 'L194A)L2'

=> s l1(4a)l2

*WARNING* - PROXIMITY OPERATOR PRECEDENCE LEVEL CONFLICTS OR IS NOT CONSIS
TENT WITH
FIELD CODE - 'AND' OPERATOR ASSUMED 'L1(4A)L2'
L5         724 L1(4A)L2

=> s l1(4a)quantitat?

         80067 QUANTITAT?
L6           0 L1(4A)QUANTITAT?

=> s quantitat?(4a)target

         80067 QUANTITAT?
        126635 TARGET
L7         416 QUANTITAT?(4A)TARGET

=> s l7 and (dna or rna or nucleic acid)

         33084 DNA
         19731 RNA
         23666 NUCLEIC
        467480 ACID
         18913 NUCLEIC ACID
                 (NUCLEIC(W)ACID)
L8         263 L7 AND (DNA OR RNA OR NUCLEIC ACID)

=> s quantitat?(4a)(dna or rna or nucleoc acid)

         80067 QUANTITAT?
         33084 DNA
```

```
         19731 RNA
             1 NUCLEOC
        467480 ACID
             1 NUCLEOC ACID
                 (NUCLEOC(W)ACID)
L9        1208 QUANTITAT?(4A)(DNA OR RNA OR NUCLEOC ACID)

=> s quantitat?(4a)(dna or rna or nucleic acid#)

         80067 QUANTITAT?
         33084 DNA
         19731 RNA
         23666 NUCLEIC
        480112 ACID#
         23536 NUCLEIC ACID#
                 (NUCLEIC(W)ACID#)
L10       1418 QUANTITAT?(4A)(DNA OR RNA OR NUCLEIC ACID#)

=> s l10 and l1

L11        323 L10 AND L1

=> d his


     (FILE 'USPAT' ENTERED AT 12:37:41 ON 20 SEP 1999)
L1         2073 S TRIPLE HELIX OR TRIPLEX
L2          724 S L1 AND QUANTITAT?
L3        17694 S HYBRIDIZ?
L4          617 S L2 AND L3
L5          724 S L1(4A)L2
L6            0 S L1(4A)QUANTITAT?
L7          416 S QUANTITAT?(4A)TARGET
L8          263 S L7 AND (DNA OR RNA OR NUCLEIC ACID)
L9         1208 S QUANTITAT?(4A)(DNA OR RNA OR NUCLEOC ACID)
L10        1418 S QUANTITAT?(4A)(DNA OR RNA OR NUCLEIC ACID#)
L11         323 S L10 AND L1

=> s l11 and hybridiz?

         17694 HYBRIDIZ?
L12        321 L11 AND HYBRIDIZ?

=> s l12 and densitom?

         10134 DENSITOM?
L13         21 L12 AND DENSITOM?

=> d l13 1-21


1.  5,948,888, Sep. 7, 1999, Neural thread protein gene expression and
detection of Alzheimer's disease; Suzanne de la Monte, et al., 530/350;
435/69.1, 71.2, 252.3, 252.33, 320.1; 530/839 [IMAGE AVAILABLE]

2.  5,948,634, Sep. 7, 1999, Neural thread protein gene expression and
detection of alzheimer's disease; Suzanne de la Monte, et al., 435/69.1,
252.3, 252.8, 320.1; 536/23.1, 23.5 [IMAGE AVAILABLE]

3.  5,914,269, Jun. 22, 1999, Oligonucleotide inhibition of epidermal
growth factor receptor expression; C. Frank Bennett, et al., 435/375, 6;
514/44; 536/23.1, 23.2, 24.1, 24.5 [IMAGE AVAILABLE]

4.  5,874,285, Feb. 23, 1999, Polynucleotide encoding a novel human
nm23-like protein; Olga Bandman, et al., 435/252.3, 320.1; 536/23.1
```

[IMAGE AVAILABLE]

5. 5,874,218, Feb. 23, 1999, Method for detecting a target compound in a substance using a nucleic acid ligand; Dan Drolet, et al., 435/6, 91.2 [IMAGE AVAILABLE]

6. 5,869,241, Feb. 9, 1999, Method of determining DNA sequence preference of a DNA-binding molecule; Cynthia A. Edwards, et al., 435/6, 91.1, 91.2 [IMAGE AVAILABLE]

7. 5,846,720, Dec. 8, 1998, Methods of determining chemicals that modulate expression of genes associated with cardiovascular disease; J. Gordon Foulkes, et al., 435/6, 69.8, 91.5, 320.1 [IMAGE AVAILABLE]

8. 5,830,670, Nov. 3, 1998, Neural thread protein gene expression and detection of Alzheimer's disease; Suzanne de la Monte, et al., 435/7.2, 7.1, 7.92, 40.52; 436/63 [IMAGE AVAILABLE]

9. 5,744,131, Apr. 28, 1998, Sequence-directed DNA-binding molecules compositions and methods; Cynthia A. Edwards, et al., 424/78.08; 436/501; 514/1 [IMAGE AVAILABLE]

10. 5,738,990, Apr. 14, 1998, Sequence-directed DNA-binding molecules compositions and methods; Cynthia A. Edwards, et al., 435/6, 69.1, 320.1; 536/24.1 [IMAGE AVAILABLE]

11. 5,726,014, Mar. 10, 1998, Screening assay for the detection of DNA-binding molecules; Cynthia A. Edwards, et al., 435/6, 91.2; 436/501 [IMAGE AVAILABLE]

12. 5,716,780, Feb. 10, 1998, Method of constructing sequence-specific DNA-binding molecules; Cynthia A. Edwards, et al., 435/6; 436/501 [IMAGE AVAILABLE]

13. 5,693,463, Dec. 2, 1997, Method of ordering sequence binding preferences of a DNA-binding molecule; Cynthia A. Edwards, et al., 435/6, 7.23; 536/23.1 [IMAGE AVAILABLE]

14. 5,641,625, Jun. 24, 1997, Cleaving double-stranded DNA with peptide nucleic acids; David J. Ecker, et al., 435/6; 536/24.3 [IMAGE AVAILABLE]

15. 5,624,803, Apr. 29, 1997, In vivo oligonucleotide generator, and methods of testing the binding affinity of **triplex** forming oligonucleotides derived therefrom; Sarah B. Noonberg, et al., 435/6, 91.1, 320.1; 536/24.1 [IMAGE AVAILABLE]

16. 5,612,215, Mar. 18, 1997, Stromelysin targeted ribozymes; Kenneth G. Draper, et al., 435/366, 6, 91.31, 320.1, 325; 514/44; 536/23.1, 23.2, 24.5 [IMAGE AVAILABLE]

17. 5,580,722, Dec. 3, 1996, Methods of determining chemicals that modulate transcriptionally expression of genes associated with cardiovascular disease; J. Gordon Foulkes, et al., 435/6, 91.1, 91.2 [IMAGE AVAILABLE]

18. 5,578,444, Nov. 26, 1996, Sequence-directed DNA-binding molecules compositions and methods; Cynthia A. Edwards, et al., 435/6, 7.23; 536/23.1 [IMAGE AVAILABLE]

19. 5,539,082, Jul. 23, 1996, Peptide nucleic acids; Peter E. Nielsen, et al., 530/300; 536/18.7, 24.3; 544/242, 264 [IMAGE AVAILABLE]

20. 5,198,346, Mar. 30, 1993, Generation and selection of novel DNA-binding proteins and polypeptides; Robert C. Ladner, et al., 435/69.1, 252.3, 320.1, 489 [IMAGE AVAILABLE]

21. 5,096,815, Mar. 17, 1992, Generation and selection of novel DNA-binding proteins and polypeptides; Robert C. Ladner, et al., 435/69.1, 252.3, 320.1 [IMAGE AVAILABLE]

=> d his

(FILE 'USPAT' ENTERED AT 12:37:41 ON 20 SEP 1999)

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L1          2073 S TRIPLE HELIX OR TRIPLEX
L2           724 S L1 AND QUANTITAT?
L3         17694 S HYBRIDIZ?
L4           617 S L2 AND L3
L5           724 S L1(4A)L2
L6             0 S L1(4A)QUANTITAT?
L7           416 S QUANTITAT?(4A)TARGET
L8           263 S L7 AND (DNA OR RNA OR NUCLEIC ACID)
L9          1208 S QUANTITAT?(4A)(DNA OR RNA OR NUCLEOC ACID)
L10         1418 S QUANTITAT?(4A)(DNA OR RNA OR NUCLEIC ACID#)
L11          323 S L10 AND L1
L12          321 S L11 AND HYBRIDIZ?
L13           21 S L12 AND DENSITOM?
```

=> d l13 15 ab

US PAT NO: 5,624,803 [IMAGE AVAILABLE] L13: 15 of 21

ABSTRACT:
The present invention encompasses improved methods and materials for the delivering of antisense, **triplex**, and/or ribozyme oligonucleotides intracellularly, and RNA polymerase III-based constructs termed "oligonucleotide generators" to accomplish the delivery of oligonucleotides. Also encompassed by the present invention are methods for screening oligonucleotide sequences that are candidates for **triplex** formation.

=> d l13 5 kwic

US PAT NO: 5,874,218 [IMAGE AVAILABLE] L13: 5 of 21

SUMMARY:

BSUM(16)

In . . . of oligonucleotide probes is used to specifically target genomic complementary base sequences in techniques such as Southern blotting, in situ **hybridization** and polymerase chain reaction (PCR)-based amplifications. However, in these processes information stored in an oligonucleotide is only generally used to. . .

DRAWING DESC:

DRWD(4)

FIG. . . . the plot of density versus VEGF.sub.165 concentration obtained from the blot shown in FIG. 2 as read on a Personal **Densitometer** 100 Minute Exposures. Density on the film is proportional to the amount of VEGF.sub.165 loaded. Thus the technique can be. . .

DRAWING DESC:

DRWD(8)

FIG. 7 depicts the **quantitation** of radiolabeled **nucleic acid** ligand as it correlates with the concentration of hCG in the blot assay.

DETDESC:

DETD(2)

This . . . a polynucleotide that binds to the nucleic acid ligand through a mechanism which predominantly depends on Watson/Crick base pairing or **triple helix** binding, and wherein the nucleic acid ligand is not a nucleic acid having the known physiological function of being bound. . .

DETDESC:

DETD(50)

Human . . . the membrane was wrapped in plastic wrap and exposed to film (BioMax; Kodak) for 10 minutes. After developing the film, **densitometry** was performed using a Personal **Densitometer** (Molecular Dynamics), according to the manufacturers directions. Data were fit to a one site binding hyperbola model using GraphPad Prism. .

DETDESC:

DETD(51)

Shown . . . on the membrane. Such nonspecific binding is sometimes observed for antibodies as well. FIG. 3 shows the result of a **densitometry** scan of the film shown in FIG. 2. The shape of this curve was a typical saturation binding isotherm and. . .

=> d l13 1-21

1. 5,948,888, Sep. 7, 1999, Neural thread protein gene expression and detection of Alzheimer's disease; Suzanne de la Monte, et al., 530/350; 435/69.1, 71.2, 252.3, 252.33, 320.1; 530/839 [IMAGE AVAILABLE]

2. 5,948,634, Sep. 7, 1999, Neural thread protein gene.expression and detection of alzheimer's disease; Suzanne de la Monte, et al., 435/69.1, 252.3, 252.8, 320.1; 536/23.1, 23.5 [IMAGE AVAILABLE]

3. 5,914,269, Jun. 22, 1999, Oligonucleotide inhibition of epidermal growth factor receptor expression; C. Frank Bennett, et al., 435/375, 6; 514/44; 536/23.1, 23.2, 24.1, 24.5 [IMAGE AVAILABLE]

4. 5,874,285, Feb. 23, 1999, Polynucleotide encoding a novel human nm23-like protein; Olga Bandman, et al., 435/252.3, 320.1; 536/23.1 [IMAGE AVAILABLE]

5. 5,874,218, Feb. 23, 1999, Method for detecting a target compound in a substance using a nucleic acid ligand; Dan Drolet, et al., 435/6, 91.2 [IMAGE AVAILABLE]

6. 5,869,241, Feb. 9, 1999, Method of determining DNA sequence preference of a DNA-binding molecule; Cynthia A. Edwards, et al., 435/6, 91.1, 91.2 [IMAGE AVAILABLE]

7. 5,846,720, Dec. 8, 1998, Methods of determining chemicals that modulate expression of genes associated with cardiovascular disease; J. Gordon Foulkes, et al., 435/6, 69.8, 91.5, 320.1 [IMAGE AVAILABLE]

8. 5,830,670, Nov. 3, 1998, Neural thread protein gene expression and detection of Alzheimer's disease; Suzanne de la Monte, et al., 435/7.2, 7.1, 7.92, 40.52; 436/63 [IMAGE AVAILABLE]

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10. 5,738,990, Apr. 14, 1998, Sequence-directed DNA-binding molecules compositions and methods; Cynthia A. Edwards, et al., 435/6, 69.1, 320.1; 536/24.1 [IMAGE AVAILABLE]

11. 5,726,014, Mar. 10, 1998, Screening assay for the detection of DNA-binding molecules; Cynthia A. Edwards, et al., 435/6, 91.2; 436/501 [IMAGE AVAILABLE]

12. 5,716,780, Feb. 10, 1998, Method of constructing sequence-specific DNA-binding molecules; Cynthia A. Edwards, et al., 435/6; 436/501 [IMAGE AVAILABLE]

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14. 5,641,625, Jun. 24, 1997, Cleaving double-stranded DNA with peptide nucleic acids; David J. Ecker, et al., 435/6; 536/24.3 [IMAGE AVAILABLE]

15. 5,624,803, Apr. 29, 1997, In vivo oligonucleotide generator, and methods of testing the binding affinity of **triplex** forming oligonucleotides derived therefrom; Sarah B. Noonberg, et al., 435/6, 91.1, 320.1; 536/24.1 [IMAGE AVAILABLE]

16. 5,612,215, Mar. 18, 1997, Stromelysin targeted ribozymes; Kenneth G. Draper, et al., 435/366, 6, 91.31, 320.1, 325; 514/44; 536/23.1, 23.2, 24.5 [IMAGE AVAILABLE]

17. 5,580,722, Dec. 3, 1996, Methods of determining chemicals that modulate transcriptionally expression of genes associated with cardiovascular disease; J. Gordon Foulkes, et al., 435/6, 91.1, 91.2 [IMAGE AVAILABLE]

18. 5,578,444, Nov. 26, 1996, Sequence-directed DNA-binding molecules compositions and methods; Cynthia A. Edwards, et al., 435/6, 7.23; 536/23.1 [IMAGE AVAILABLE]

19. 5,539,082, Jul. 23, 1996, Peptide nucleic acids; Peter E. Nielsen, et al., 530/300; 536/18.7, 24.3; 544/242, 264 [IMAGE AVAILABLE]

20. 5,198,346, Mar. 30, 1993, Generation and selection of novel DNA-binding proteins and polypeptides; Robert C. Ladner, et al., 435/69.1, 252.3, 320.1, 489 [IMAGE AVAILABLE]

21. 5,096,815, Mar. 17, 1992, Generation and selection of novel DNA-binding proteins and polypeptides; Robert C. Ladner, et al., 435/69.1, 252.3, 320.1 [IMAGE AVAILABLE]

=> d l13 18 ab,kwic

US PAT NO: 5,578,444 [IMAGE AVAILABLE] L13: 18 of 21

ABSTRACT:
The present invention defines a DNA:protein-binding assay useful for screening libraries of synthetic or biological compounds for their ability to bind DNA test sequences. The assay is versatile in that any

number of test sequences can be tested by placing the test sequence adjacent to a defined protein binding screening sequence. Binding of molecules to these test sequence changes the binding characteristics of the protein molecule to its cognate binding sequence. When such a molecule binds the test sequence the equilibrium of the DNA:protein complexes is disturbed, generating changes in the concentration of free DNA probe. Numerous exemplary target test sequences (SEQ ID NO:1 to SEQ ID NO:600) are set forth. The assay of the present invention is also useful to characterize the preferred binding sequences of any selected DNA-binding molecule.

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=> d his


     (FILE 'USPAT' ENTERED AT 12:37:41 ON 20 SEP 1999)
L1          2073 S TRIPLE HELIX OR TRIPLEX
L2           724 S L1 AND QUANTITAT?
L3         17694 S HYBRIDIZ?
L4           617 S L2 AND L3
L5           724 S L1(4A)L2
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L8           263 S L7 AND (DNA OR RNA OR NUCLEIC ACID)
L9          1208 S QUANTITAT?(4A)(DNA OR RNA OR NUCLEOC ACID)
L10         1418 S QUANTITAT?(4A)(DNA OR RNA OR NUCLEIC ACID#)
L11          323 S L10 AND L1
L12          321 S L11 AND HYBRIDIZ?
L13           21 S L12 AND DENSITOM?

=> s quantitat?(4a)(dna or rna or nucleic acid#)(4a)(densitom?)

          80067 QUANTITAT?
          33084 DNA
          19731 RNA
          23666 NUCLEIC
         480112 ACID#
          23536 NUCLEIC ACID#
                  (NUCLEIC(W)ACID#)
          10134 DENSITOM?
L14          17 QUANTITAT?(4A)(DNA OR RNA OR NUCLEIC ACID#)(4A)(DENSITOM?)

=> d l14 1-17
```

1. 5,906,976, May 25, 1999, Method and composition for treating neuronal degeneration; Lily Vardimon, 514/12; 424/94.1; 514/2, 26 [IMAGE AVAILABLE]

2. 5,871,958, Feb. 16, 1999, Mutant rev genes encoding transdominant repressors of HIV replication; Bryan R. Cullen, 435/69.1, 91.1, 320.1; 536/23.72 [IMAGE AVAILABLE]

3. 5,871,909, Feb. 16, 1999, Human cellular retinoic acid binding protein I and II DNA and methods of use; Anders .ANG.strom, et al., 435/6, 320.1; 536/23.1, 23.5 [IMAGE AVAILABLE]

4. 5,869,241, Feb. 9, 1999, Method of determining DNA sequence preference of a DNA-binding molecule; Cynthia A. Edwards, et al., 435/6, 91.1, 91.2 [IMAGE AVAILABLE]

5. 5,811,281, Sep. 22, 1998, Immortalized intestinal epithelial cell lines; Andrea Quaroni, et al., 435/353, 320.1, 467 [IMAGE AVAILABLE]

6. 5,798,258, Aug. 25, 1998, Cart protein and DNA encoding therefor; James O. Douglass, 435/252.3, 69.1, 320.1, 325; 530/350; 536/23.1 [IMAGE

AVAILABLE]

7. 5,756,348, May 26, 1998, DNA encoding a glycine transporter and uses thereof; Kelli E. Smith, et al., 435/325, 252.33, 255.1, 320.1; 536/23.5 [IMAGE AVAILABLE]

8. 5,753,437, May 19, 1998, Method of diagnosing cancer susceptibility or metastatic potential; Patricia S. Steeg, et al., 435/6, 91.1, 91.2; 536/23.5, 24.31 [IMAGE AVAILABLE]

9. 5,747,650, May 5, 1998, P53AS protein and antibody therefor; Molly F. Kulesz-Martin, 530/387.7, 387.1, 388.8, 389.1, 389.2 [IMAGE AVAILABLE]

10. 5,744,492, Apr. 28, 1998, Method for inhibiting angiogenesis; Elise C. Kohn, et al., 514/359, 356, 648, 650 [IMAGE AVAILABLE]

11. 5,726,014, Mar. 10, 1998, Screening assay for the detection of DNA-binding molecules; Cynthia A. Edwards, et al., 435/6, 91.2; 436/501 [IMAGE AVAILABLE]

12. 5,654,137, Aug. 5, 1997, Human CRABP-I and CRABP-II; Anders Astrom, et al., 435/5, 6, 371 [IMAGE AVAILABLE]

13. 5,643,761, Jul. 1, 1997, Method for generating a subtracted cDNA library and uses of the generated library; Paul B. Fisher, et al., 435/91.1, 6, 69.1, 91.2, 488, 489, 810; 436/501; 536/23.1, 24.1, 24.3, 24.31, 24.32, 24.33 [IMAGE AVAILABLE]

14. 5,616,466, Apr. 1, 1997, Ribozyme-mediated inhibition of bovine leukemia virus; Glenn H. Cantor, et al., 435/6, 91.31, 320.1, 455; 514/44; 536/23.1, 23.2, 24.5 [IMAGE AVAILABLE]

15. 5,612,215, Mar. 18, 1997, Stromelysin targeted ribozymes; Kenneth G. Draper, et al., 435/366, 6, 91.31, 320.1, 325; 514/44; 536/23.1, 23.2, 24.5 [IMAGE AVAILABLE]

16. 5,578,444, Nov. 26, 1996, Sequence-directed DNA-binding molecules compositions and methods; Cynthia A. Edwards, et al., 435/6, 7.23; 536/23.1 [IMAGE AVAILABLE]

17. 5,157,115, Oct. 20, 1992, Regulation of expression; Tadatsugu Taniguchi, 536/23.51, 24.32, 24.5 [IMAGE AVAILABLE]

=> d l14 17 kwic

US PAT NO: 5,157,115 [IMAGE AVAILABLE] L14: 17 of 17

DETDESC:

DETD(42)

FIG. . . . IL-2 DNA segment as described above. The DNAs were exactly the same as described above. The efficacy of formation of factor-**DNA** complexes were **quantitated** by **densitometric** analysis of the autoradiogram. Complex formation in the absence of competitor DNAs was taken as 100%.

=> d l14 15 kwic

US PAT NO: 5,612,215 [IMAGE AVAILABLE] L14: 15 of 17

=> d l14 15 kwic

US PAT NO: 5,612,215 [IMAGE AVAILABLE] L14: 15 of 17

=> d l14 13 kwic

US PAT NO: 5,643,761 [IMAGE AVAILABLE] L14: 13 of 17

DETDESC:

DETD(366)

(iv) . . . analyzed by Northern hybridization and probing with the different mda genes or GAPDH (14,49,55). Radioautograms will be scanned using a **densitometer** to **quantitate** cellular **RNA** levels (55). These studies will indicate if IFN-.beta.+MEZ can alter the stability, i.e., the half-life, of any of the mda. . .

=> d l14 1-12 kwic

US PAT NO: 5,906,976 [IMAGE AVAILABLE] L14: 1 of 17

DETDESC:

DETD(50)

Quantitation . . . by electrophoresis in 1.8% agarose gels. The gels were stained with ethidium bromide, visualized by ultraviolet (UV) light and photographed. **DNA** fragmentation was **quantitated** by **densitometric** scanning of the pictures.

US PAT NO: 5,871,958 [IMAGE AVAILABLE] L14: 2 of 17

DETDESC:

DETD(167)

At . . . probe fragment while unspliced (U) tat mRNA is predicted to rescue a 506 nt fragment. The relative level of unspliced **RNA** in each lane was **quantitated** by **densitometry** using an LKB soft laser scanner. The results, as visualized in FIG. 11B, are: Lane 2: 14% unspliced; Lane 3:. . .

US PAT NO: 5,871,909 [IMAGE AVAILABLE] L14: 3 of 17

DRAWING DESC:

DRWD(5)

FIG. 4 is a bar graph of the **RNA** blot hybridization results (**quantitated** by laser **densitometry**) of nine independent experiments involving five dermal fibroblast lines prepared from three individuals and three diploid human lung fibroblast lines.. . .

DETDESC:

DETD(45)

Treatment . . . as described above and three diploid human lung fibroblast lines (LL47, CCD-18Lu, and CCD-16Lu). RNA blot hybridizations (20 .mu.g total **RNA**/lane) were **quantitated** by laser **densitometry** and normalized to the control gene, cyclophilin, as described in Elder, J. T. et al., J. Invest. Dermatol. 94:19-25 (1990).. . .

US PAT NO: 5,869,241 [IMAGE AVAILABLE] L14: 4 of 17

US PAT NO: 5,811,281 [IMAGE AVAILABLE] L14: 5 of 17

DETDESC:

DETD(20)

Total . . . rRNA probe was hybridized to the filters and then removed by stringent washing in 1.times.SSC, 0.1% SDS at 65.degree. C. **RNA** was **quantitated** using a **densitometer**; the amounts of keratin and actin RNA were standardized to the 18S rRNA.

US PAT NO: 5,798,258 [IMAGE AVAILABLE] L14: 6 of 17

DETDESC:

DETD(46)

To . . . membranes were exposed multiple times to Kodak XAR-5 film in order to obtain a range of hybridization signal intensities for semi-**quantitative densitometric** analysis. For each **RNA** sample, autoradiographic signals within the linear range of film sensitivity were digitized using an X-Ray Scanner Corp. model MSF300ZS laser. . .

US PAT NO: 5,756,348 [IMAGE AVAILABLE] L14: 7 of 17

DRAWING DESC:

DRWD(7)

Total . . . the blot with a cDNA probe, designated p1B15, against cyclophilin. Similar results were obtained by using a probe to .beta.-actin. **Quantitation** of the **RNA** blot was performed by **densitometer** scanning.

US PAT NO: 5,753,437 [IMAGE AVAILABLE] L14: 8 of 17

DETDESC:

DETD(70)

(1) . . . 2, 1, 0.5 and 0.25xSSC, 0.1% SDS (w/v), 1 mM EDTA at 55.degree. C., and exposed to X-ray film. NM23 **RNA** levels can be **quantitated** by **densitometry** or other means. Other methods for determining NM23 RNA levels, such as slot blots, RNase protection or dot blots, may. . .

US PAT NO: 5,747,650 [IMAGE AVAILABLE] L14: 9 of 17

DETDESC:

DETD(125)

RNA . . . (Amersham, Arlington Heights, Ill.). 32P-labeled probe was used at a final concentration of 1 to 2.times.10.sup.6 cpm/ml. Differences in p53 **RNA** abundance were **quantitated** by **densitometry** of exposed films (Fastscan computing densitometer, Molecular Dynamics, Sunnyvale, Calif.) after adjustment for 7S RNA.

US PAT NO: 5,744,492 [IMAGE AVAILABLE] L14: 10 of 17

DETDESC:

DETD(66)

HUVECs . . . conditions then exposed to film. After removal of MMP-2 probe, blots were rehybridized with .beta.-actin probe for quantitation of the **RNA** load. Results were **quantitated** by **densitometric** evaluation of the autoradiographs.

US PAT NO: 5,726,014 [IMAGE AVAILABLE] L14: 11 of 17

US PAT NO: 5,654,137 [IMAGE AVAILABLE] L14: 12 of 17

DRAWING DESC:

DRWD(5)

FIG. 4 is a bar graph of the **RNA** blot hybridization results (**quantitated** by laser **densitometry**) of nine independent experiments Involving five dermal fibroblast lines prepared from three individuals and three diploid human lung fibroblast lines.. . .

DETDESC:

DETD(43)

Treatment . . . as described above and three diploid human lung fibroblast lines (LL47, CCD-18Lu, and CCD-16Lu). RNA blot hybridizations (20 .mu.g total **RNA**/lane) were **quantitated** by laser **densitometry** and normalized to the control gene, cyclophilin, as described in Elder, J. T. et al., J. Invest. Dermatol. 94:19-25 (1990).. . .

=> s dna(4a)quantitat?(densitom?)

MISSING OPERATOR 'QUANTITAT?(DENSITOM?'

=> s dna(4a)quantitat?(4a)(densitom?)

```
         33084 DNA
         80067 QUANTITAT?
         10134 DENSITOM?
L15          2 DNA(4A)QUANTITAT?(4A)(DENSITOM?)
```

=> d l15 1-2 kwic

US PAT NO: 5,906,976 [IMAGE AVAILABLE] L15: 1 of 2

DETDESC:

DETD(50)

Quantitation . . . by electrophoresis in 1.8% agarose gels. The gels were stained with ethidium bromide, visualized by ultraviolet (UV) light and photographed. **DNA** fragmentation was **quantitated** by **densitometric** scanning of the pictures.

US PAT NO: 5,157,115 [IMAGE AVAILABLE] L15: 2 of 2

DETDESC:

DETD(42)

FIG. . . . IL-2 DNA segment as described above. The DNAs were exactly the same as described above. The efficacy of formation of factor-**DNA**

complexes were quantitated by densitometric analysis of the autoradiogram. Complex formation in the absence of competitor DNAs was taken as 100%.

L17 ANSWER 1 OF 8 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1998:256659 BIOSIS
DOCUMENT NUMBER: PREV199800256659
TITLE: Analysis of various sequence-specific **triplexes** by electron and atomic force microscopies.
AUTHOR(S): Cherny, Dimitry I. (1); Fourcade, Alain; Svinarchuk, Fedro;
Nielsen, Peter E.; Malvy, Claude; Delain, Etienne
CORPORATE SOURCE: (1) Lab. Microscopie Cellularie Molecularie, URA 147, CNRS,
Inst. Gustave-Roussy, rue Camillie Desmoulin, F-94805 Villejuif France
STIC SOURCE: Biophysical Journal, (Feb., 1998) Vol. 74, No. 2 PART 1, pp. 1015-1023.
ISSN: 0006-3495.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Sequence-specific interactions of 20-mer G,A-containing **triple helix**-forming oligonucleotides (TFOs) and bis-**PNAs** (**peptide nucleic acids**) with double-**stranded** DNA was visualized by electron (EM) and atomic force (AFM) microscopies. **Triplexes** formed by biotinylated TFOs are easily detected by both EM and AFM in which streptavidin is a marker. AFM images of the unlabeled **triplex** within a long plasmid DNA show a apprx0.4-nm height increment of the double **helix** within the target site position. TFOs conjugated to a 74-nt-long oligonucleotide forming a 33-bp-long hairpin form extremely stable **triplexes** with the target site that are readily imaged by both EM and AFM as protruding DNA. The **short** duplex protrudes in a perpendicular direction relative to the double **helix** axis, either in the plane of the support or out of it. In the latter case, the apparent height of the protrusion is apprx 1.5 nm, when that of the **triplex** site is increased by 0.3-0.4 nm. **Triplex** formation by bis-**PNA**, in which two decamers of **PNA** are connected via a flexible linker, causes deformations of the double **helix** at the target site, which is readily detected as kinks by both EM and AFM. Moreover, AFM shows that these kinks are often accompanied by an increase in the DNA apparent height of apprx 35%. This work shows the first direct visualization of sequence-specific interaction of TFOs and **PNAs**, with their target sequences within long plasmid DNAs, through the measurements of the apparent height of the DNA double **helix** by AFM.

=> d ibib abs 1-7

L17 ANSWER 1 OF 8 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1998:256659 BIOSIS
DOCUMENT NUMBER: PREV199800256659
TITLE: Analysis of various sequence-specific **triplexes** by electron and atomic force microscopies.
AUTHOR(S): Cherny, Dimitry I. (1); Fourcade, Alain; Svinarchuk, Fedro;
Nielsen, Peter E.; Malvy, Claude; Delain, Etienne
CORPORATE SOURCE: (1) Lab. Microscopie Cellularie Molecularie, URA 147, CNRS,

Inst. Gustave-Roussy, rue Camillie Desmoulin, F-94805 Villejuif France
SOURCE: Biophysical Journal, (Feb., 1998) Vol. 74, No. 2 PART 1, pp. 1015-1023.
ISSN: 0006-3495.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Sequence-specific interactions of 20-mer G,A-containing **triple helix**-forming oligonucleotides (TFOs) and bis-**PNAs** (**peptide nucleic acids**) with double-**stranded** DNA was visualized by electron (EM) and atomic force (AFM) microscopies. **Triplexes** formed by biotinylated TFOs are easily detected by both EM and AFM in which streptavidin is a marker. AFM images of the unlabeled **triplex** within a long plasmid DNA show a apprx0.4-nm height increment of the double **helix** within the target site position. TFOs conjugated to a 74-nt-long oligonucleotide forming a 33-bp-long hairpin form extremely stable **triplexes** with the target site that are readily imaged by both EM and AFM as protruding DNA. The **short** duplex protrudes in a perpendicular direction relative to the double **helix** axis, either in the plane of the support or out of it. In the latter case, the apparent height of the protrusion is apprx 1.5 nm, when that of the **triplex** site is increased by 0.3-0.4 nm. **Triplex** formation by bis-**PNA**, in which two decamers of **PNA** are connected via a flexible linker, causes deformations of the double **helix** at the target site, which is readily detected as kinks by both EM and AFM. Moreover, AFM shows that these kinks are often accompanied by an increase in the DNA apparent height of apprx 35%. This work shows the first direct visualization of sequence-specific interaction of TFOs and **PNAs**, with their target sequences within long plasmid DNAs, through the measurements of the apparent height of the DNA double **helix** by AFM.

L17 ANSWER 2 OF 8 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1995:102057 BIOSIS
DOCUMENT NUMBER: PREV199598116357
TITLE: Electron microscopy mapping of oligopurine tracts in duplex DNA by **peptide nucleic acid** targeting.
AUTHOR(S): Demidov, Vadim V.; Cherny, Dmitry I.; Kurakin, Alexey V.; Yavnilovich, Michael V.; Malkov, Vladislav A.; Frank-Kamenetskii, Maxim D.; Sonnichsen, Soren H.; Nielsen, Peter E. (1)
CORPORATE SOURCE: (1) Cent. Biomol. Recognition, IMBG Dep. B, Panum Inst., Blegdamsvej 3c, DK-2200 Copenhagen N Denmark
SOURCE: Nucleic Acids Research, (1994) Vol. 22, No. 24, pp. 5218-5222.
ISSN: 0305-1048.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Biotinylated homopyrimidine decamer **peptide nucleic acids** (**PNAs**) are shown to form sequence-specific and stable complexes with complementary oligopurine targets in linear double-**stranded** DNA. The noncovalent complexes are visualized by electron microscopy (EM) without chemical fixation using streptavidin as an EM marker. The **triplex** stoichiometry of the **PNA**-DNA complexes (two **PNA** molecules presumably binding by Watson-Crick and Hoogsteen pairing with one of the **strands** of the duplex DNA) is indicated by the appearance of two streptavidin 'beads' per target site in some micrographs, and is also supported by the formation of two retardation bands in a gel shift assay. Quantitative analysis of the

positions of the streptavidin 'beads' revealed that under optimized conditions **PNA**-DNA complexes are preferably formed with the fully complementary target. An increase in either the **PNA** concentration or the incubation time leads to binding at sites containing one or two mismatches. Our results demonstrate that biotinylated **PNAs** can be used for EM mapping of **short** targets in duplex DNA.

L17 ANSWER 3 OF 8 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1994:17430 BIOSIS
DOCUMENT NUMBER: PREV199497030430
TITLE: **Peptide nucleic acid** (**PNA**) conformation and polymorphism in **PNA**-DNA and **PNA**-RNA hybrids.
AUTHOR(S): Almarsson, Orn; Bruice, Thomas C.
CORPORATE SOURCE: Dep. Chem., Univ. California Santa Barbara, Santa Barbara, CA 93106 USA
SOURCE: Proceedings of the National Academy of Sciences of the United States of America, (1993) Vol. 90, No. 20, pp. 9542-9546.
ISSN: 0027-8424.
DOCUMENT TYPE: Article
LANGUAGE: English
AB Two hydrogen-bonding motifs have been proposed to account for the extraordinary stability of polyamide "**peptide**" **nucleic acid** (**PNA**) hybrids with nucleic acids. These interresidue- and intraresidue-hydrogen-bond motifs were investigated by molecular mechanics calculations. Energy-minimized structures of Watson-Crick base-paired decameric duplexes of **PNA** with A-, B-, and Z-DNA and A-RNA polymorphs indicate that the inherent stability of the complementary **PNA** helical structures is derived from interresidue, rather than from intraresidue, hydrogen bonds in an hybrids studied. Intraresidue-hydrogen-bond lengths are consistently longer than interresidue hydrogen bonds. Helical **strand** stability with interresidue hydrogen bond stabilization follows the order: B-(DNA cntdot **PNA**) gt A-(DNA cntdot **PNA**) simeq A-RNA cntdot **PNA** gt Z-(DNA cntdot **PNA**). In the **triplex** hybrids A-(RNA cntdot **PNA**-2) and B-(DNA cntdot **PNA**-2), differences between stabilities of the two decamers of thyminyl **PNA** with lysine amide attached to the C terminus (pnaT)-10 **strands** are **small**. The Hoogsteen (pnaT)-10 **strands** are of slightly higher potential energy than are the Watson-Crick (pnaT)-10 **strands**. Antiparallel arrangement of **PNAs** in the **triplex** is slightly favored over the parallel arrangement based on the calculations. Examination by molecular mechanics of the **PNA**-DNA analogue of the NMR-derived structure for the B-double-**stranded** DNA dodecamer d(CGCAAATTTGCG)-2 in solution suggests that use of an bases of the genetic alphabet should be possible without loss of the specific interresidue-hydrogen-bonding pattern within the **PNA strand**.

L17 ANSWER 4 OF 8 BIOSIS COPYRIGHT 1999 BIOSIS
ACCESSION NUMBER: 1993:428636 BIOSIS
DOCUMENT NUMBER: PREV199396083261
TITLE: Right-handed **triplex** formed between **peptide nucleic acid** **PNA**-T-8 and poly(dA) shown by linear and circular dichroism spectroscopy.
AUTHOR(S): Kim, Seog K.; Nielsen, Peter E. (1); Egholm, Michael; Buchardt, Ole; Berg, Rolf H.; Norden, Bengt
CORPORATE SOURCE: (1) Dep. Biochem. B, Panum Inst., University Copenhagen, Blegdamsvej 3, DK-2200 Copenhagen N Denmark
SOURCE: Journal of the American Chemical Society, (1993) Vol. 115, No. 15, pp. 6477-6481.

ISSN: 0002-7863.
DOCUMENT TYPE: Article
LANGUAGE: English
AB The binding of an eightmer of **peptide nucleic acid**, H-T-8-Lys-NH-2 (=**PNA**-T-8), to a polynucleotide, poly(dA), was studied by flow linear dichroism (LD) and circular dichroism (CD) spectroscopy. Whereas the single **stranded** DNA, due to its high flexibility, does not display any measurable LD signal when subjected to shear flow, the complex with **PNA** does. A titration shows that saturation occurs at a stoichiometry of two **PNA** thymine bases per DNA adenine base, indicating the formation of a **triplex** **PNA**-2-DNA complex. The persistence length of the adduct remains **small** up to relatively high stoichiometries (above 1:1 T:A) indicating that no significant amounts of **PNA**:DNA duplex are formed. Instead **triplex** stretches seem to form surrounded by flexible parts of single **stranded** poly(dA). Upon approaching the stoichiometry 2:1 of T:A the LD increases dramatically demonstrating that the stiffness of the **PNA**-DNA **triplex** arises from base-base contacts preventing bending of the chain. It is also inferred that the main stiffness of duplex DNA very probably has a similar origin and is not primarily a result of the increased phosphate-phosphate repulsion. Circular dichroism spectra support the conclusion that a **triplex** is formed as the only **PNA**-DNA complex and that it is a right-handed **helix**. The wavelength dependence of the reduced linear dichroism shows that the inclination of the bases from perpendicularity relative to the **helix** axis is **small**. The base conformation of the poly(dA) (**PNA**-T-8)-2 **triplex** is very similar to that of the conventional poly(dA) (poly(dT))-2 **triplex**.

L17 ANSWER 5 OF 8 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1999:147810 CAPLUS
DOCUMENT NUMBER: 130:178336
TITLE: Detection of nucleic acid targets with **small** Hoogsteen bond-forming **peptide nucleic acids** and larger Watson-Crick bond-forming oligonucleotides
INVENTOR(S): Naesby, Michael
PATENT ASSIGNEE(S): Boehringer Mannheim GmbH, Germany
SOURCE: Eur. Pat. Appl., 28 pp.
CODEN: EPXXDW
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
EP 897991	A2	19990224	EP 98-115582	19980819
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
JP 11127876	A2	19990518	JP 98-235065	19980821
PRIORITY APPLN. INFO.:			EP 97-114512	19970822

AB A method for the detn. of nucleic acids which is highly specific and simple comprises the formation of a **triple stranded** binding complex including two sep., different probe mols. and detecting the formation of the complex via the inclusion of one of the probes. The method can be used to differentiate between nucleic acids having a single base difference in sequence. The invention is based on the observation that the interaction of a target DNA with a **short** Hoogsteen-binding oligonucleotide can be stabilized by a longer Watson-Crick-binding oligonucleotide. Hybridization conditions can be arranged such that the Hoogsteen-binding oligonucleotide will only bind to

my patent application

the target in the presence of the Watson-Crick-binding oligonucleotide. Addnl., stabilization of the **triplex** structure depends on regions outside of the actual **triplex** region, i.e., a single mismatch in the duplex region destabilizes the **triplex**. The Watson-Crick-binding oligonucleotide may also be split into two probes which bind adjacent to each other. This enhances specificity and allows bases even further from the **triplex**-forming region to influence **triplex** formation. The Hoogsteen-binding oligonucleotide may be a **peptide nucleic acid**.

L17 ANSWER 6 OF 8 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:373255 CAPLUS
DOCUMENT NUMBER: 129:132651
TITLE: Molecular Dynamics Simulation of a **PNA** .cntdot.DNA.cntdot.**PNA Triple Helix** in Aqueous Solution
AUTHOR(S): Shields, George C.; Laughton, Charles A.; Orozco, Modesto
CORPORATE SOURCE: Departament de Bioquimica i Biologia Molecular Facultat de Quimica, Universitat de Barcelona, Barcelona, 08028, Spain
SOURCE: J. Am. Chem. Soc. (1998), 120(24), 5895-5904
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Mol. dynamics simulations have been used to explore the conformational flexibility of a **PNA**.cntdot.DNA.cntdot.**PNA triple helix** in aq. soln. Three 1.05 ns trajectories starting from different but reasonable conformations have been generated and analyzed in detail. All three trajectories converge within about 300 ps to produce stable and very similar conformational ensembles, which resemble the crystal structure conformation in many details. However, in contrast to the crystal structure, there is a tendency for the direct hydrogen-bonds obsd. between the amide hydrogens of the Hoogsteen-binding **PNA strand** and the phosphate oxygens of the DNA **strand** to be replaced by water-mediated hydrogen bonds, which also involve pyrimidine O2 atoms. This structural transition does not appear to weaken the **triplex** structure but alters groove widths and so may relate to the potential for recognition of such structures by other ligands (**small** mols. or proteins). Energetic anal. leads us to conclude that the reason that the hybrid **PNA**/DNA **triplex** has quite different helical characteristics from the all-DNA **triplex** is not because the addnl. flexibility imparted by the replacement of sugar-phosphate by **PNA** backbones allows motions to improve base-stacking but rather that base-stacking interactions are very similar in both types of **triplex** and the driving force comes from weak but definite conformational preferences of the **PNA strands**.

L17 ANSWER 7 OF 8 CAPLUS COPYRIGHT 1999 ACS
ACCESSION NUMBER: 1998:69136 CAPLUS
DOCUMENT NUMBER: 128:150744
TITLE: Molecular Models of Nucleic Acid **Triple Helixes**. II. **PNA** and 2'-5' Backbone Complexes
AUTHOR(S): Srinivasan, A. R.; Olson, Wilma K.
CORPORATE SOURCE: Department of Chemistry Wright-Rieman Laboratories, Rutgers The State University of New Jersey, Piscataway, NJ, 08854-8087, USA
SOURCE: J. Am. Chem. Soc. (1998), 120(3), 492-508
CODEN: JACSAT; ISSN: 0002-7863
PUBLISHER: American Chemical Society
DOCUMENT TYPE: Journal
LANGUAGE: English

AB We describe nucleic acid **triple**-helical structures contg. either amide or 2'-5' linkages, the former backbone describing the chem. of certain **peptide nucleic acids** (**PNA**). The methodol. and the starting ref. frame are the same as those described in the preceding article. Apart from evaluating the possible combinations of chain conformations that connect adjacent bases on each of the three **strands**, we have examd. the feasibility of **triplex** formation when neighboring Watson-Crick+Hoogsteen hydrogen-bonded base **triples** are displaced by **small** amts. along their **short** and long axes. The predicted **triple**-helical complexes are examd. in terms of relevant crystallog., spectroscopic, and calorimetric data. The computed models clarify why **PNA** cannot form B-like structures and also reveal

4/3,AB/1 (Item 1 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

08931760 BIOSIS NO.: 199396083261
Right-handed triplex formed between peptide nucleic acid PNA-**T-8 and poly(dA) shown by linear and circular dichroism spectroscopy.**

AUTHOR: Kim Seog K; Nielsen Peter E(a); Egholm Michael; Buchardt Ole; Berg Rolf H; Norden Bengt
AUTHOR ADDRESS: (a)Dep. Biochem. B, Panum Inst., University Copenhagen, Blegdamsvej 3, DK-2100 Copenhagen Denmark

JOURNAL: Journal of the American Chemical Society 115 (15):p6477-6481 1993
ISSN: 0002-7863
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The binding of an eightmer of **peptide nucleic acid** , H-T-8-Lys-NH-2 (=**PNA** -T-8), to a polynucleotide, poly(dA), was studied by flow linear dichroism (LD) and circular dichroism (CD) spectroscopy. Whereas the single **stranded** DNA, due to its high flexibility, does not display any **measurable** LD signal when subjected to shear flow, the complex with **PNA** does. A titration shows that saturation occurs at a stoichiometry of two **PNA** thymine bases per DNA adenine base, indicating the formation of a **triplex** **PNA** -DNA complex. The persistence **length** of the adduct remains **small** up to relatively high stoichiometries (above 1:1 T:A) indicating that no significant amounts of **PNA** :DNA duplex are formed. Instead **triplex** stretches seem to form surrounded by flexible parts of single **stranded** poly(dA). Upon approaching the stoichiometry 2:1 of T:A the LD increases dramatically demonstrating that the stiffness of the **PNA** -DNA **triplex** arises from base-base contacts preventing bending of the chain. It is also inferred that the main stiffness of duplex DNA very probably has a similar origin and is not primarily a result of the increased phosphate-phosphate repulsion. Circular dichroism spectra support the conclusion that a **triplex** is formed as the only **PNA** -DNA complex and that it is a right-handed **helix** . The wavelength dependence of the reduced linear dichroism shows that the inclination of the bases from perpendicularity relative to the **helix** axis is **small** . The base conformation of the poly(dA) (**PNA** -T-8)-2 **triplex** is very similar to that of the conventional poly(dA) (poly(dT))-2 **triplex** .

4/3,AB/2 (Item 1 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04403077 Genuine Article#: TB355 Number of References: 21
Title: INDUCED CHIRALITY IN PNA- PNA **DUPLEXES**
Author(s): WITTUNG P; ERIKSSON M; LYNG R; NIELSEN PE; NORDEN B
Corporate Source: PANUM INST,DEPT BIOCHEM B,CTR BIOMOLEC RECOGNIT,BLEGDAMSVEJ 3C/DK-2200 COPENHAGEN N//DENMARK/; PANUM INST,DEPT BIOCHEM B,CTR BIOMOLEC RECOGNIT/DK-2200 COPENHAGEN N//DENMARK/; CHALMERS UNIV TECHNOL,DEPT PHYS CHEM/S-41296 GOTHENBURG//SWEDEN/
Journal: JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, 1995, V117, N41 (OCT 18) , P10167-10173
ISSN: 0002-7863
Language: ENGLISH Document Type: ARTICLE
Abstract: Complementary peptide nucleic acids (**PNA**) form Watson-Crick base-paired helical duplexes. The preferred helicity of such a duplex is determined by a chiral amino acid attached to the C-terminus. We here show that the induced helicity, as **measured** by circular dichroism (CD), is drastically dependent on the nucleobase sequence proximal to the chiral center. Chemically linked **PNA** tetramer duplexes of all 16 combinations of the two bases proximal to a carboxy terminal lysine residue were studied by CD. We conclude that the base

next to the chiral center must be either a guanine or a cytosine for efficient stabilization of one helical sense. In case of cytosine, the subsequent base should preferably be a purine. We also show that the side chain properties of the C-terminal amino acid influence the resulting sense of helicity. The propagation **length** of induced chirality in **PNA** duplexes is found to be around 10 base pairs. Theoretical calculations of the circular dichroism for B-DNA, using the quantum mechanical matrix method of Schellman, give spectra in reasonable agreement with those found experimentally for **PNA** duplexes. The rate of helix conversion of the duplexes shows first-order kinetics with a rate constant in the range of minutes. **Shorter** duplexes are found to have lower activation energy and larger negative activation entropy for helix conversion, in agreement with a conversion mechanism in which a perfect helix is switched to the opposite handedness.

4/3,AB/3 (Item 2 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci

04029025 Genuine Article#: RB093 Number of References: 46
Title: STRUCTURAL OPTIMIZATION OF NON-NUCLEOTIDE LOOP REPLACEMENTS FOR DUPLEX AND TRIPLEX DNAS
Author(s): RUMNEY S; KOOL ET
Corporate Source: UNIV ROCHESTER,DEPT CHEM/ROCHESTER//NY/14627; UNIV ROCHESTER,DEPT CHEM/ROCHESTER//NY/14627
Journal: JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, 1995, V117, N21 (MAY 31), P5635-5646
ISSN: 0002-7863
Language: ENGLISH Document Type: ARTICLE
Abstract: Described are studies systematically exploring structural effects in the use of ethylene glycol (EG) oligomers as non-nucleotide replacements for nucleotide loops in duplex and tripler DNAs. The new structurally optimized loop replacements are more stabilizing in duplexes and triplexes than previously described EG-based linkers. A series of compounds ranging in **length** from tris(ethylene glycol) to octakis(ethylene glycol) are derivatized as monodimethoxytrityl ethers on one end and phosphoramidites on the other, to enable their incorporation into DNA **strands** by automated methods. These linker molecules span lengths ranging from 13 to 31 Angstrom in extended conformation. They are incorporated into a series of duplex-forming and tripler-forming sequences, and the stabilities of the corresponding helixes are **measured** by thermal denaturation. In the duplex series, results show that the optimum linker is the one derived from heptakis(ethylene glycol), which is longer than most previous loop replacements studied. This affords a **helix** with greater thermal stability than one with a natural T-4 loop. In the tripler series, the loop replacements were examined in four separate situations, in which the loop lies in the 5' or 3' orientation and the central purine target **strand** is **short** or extends beyond the loop. Results show that in all cases the loop derived from octakis(ethylene glycol) (EG(8)) gives the greatest stability. In the cases where the target **strand** is **short**, the EG(8)-linked probe **strands** bind with affinities in some cases greater than those with a natural pentanucleotide (T-5) loop. For the cases where the target **strand** extends beyond the linker, the EG(8)-linked **strand** is destabilized relative to an optimum T-5-bridged **strand** and affinities with the EG-linked **strands** are much lower in the 5' loop orientation than in the 3' loop orientation It is found that extension by one additional nucleotide in one of the binding domains in the EG-linked series can result in considerably greater stabilities with long target **strands**. Overall, the data show that optimum loop replacements are longer than would be expected from simple distance analysis. The results are discussed in relation to expected lengths and geometries for double and **triple** helixes. The findings will be useful in the design of synthetically modified nucleic acids for use as diagnostic probes, as biochemical tools, and as potential therapeutic agents.

4/3,AB/4 (Item 3 from file: 34)
DIALOG(R)File 34:SciSearch(R) Cited Ref Sci
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02644812 Genuine Article#: LT673 Number of References: 28
Title: RIGHT-HANDED TRIPLEX FORMED BETWEEN PEPTIDE NUCLEIC- ACID PNA -T(8) AND POLY(DA) SHOWN BY LINEAR AND CIRCULAR-DICHROISM SPECTROSCOPY
Author(s): KIM SK; NIELSEN PE; EGHOLM M; BUCHARDT O; BERG RH; NORDEN B
Corporate Source: UNIV COPENHAGEN,PANUM INST,DEPT BIOCHEM B,BLEGDAMSVEJ 3/DK-2200 COPENHAGEN//DENMARK/; UNIV COPENHAGEN,PANUM INST,DEPT BIOCHEM B,BLEGDAMSVEJ 3/DK-2200 COPENHAGEN//DENMARK/; CHALMERS UNIV TECHNOL,DEPT PHYS CHEM/S-41296 GOTHENBURG//SWEDEN/; UNIV COPENHAGEN,HC ORSTED INST,DEPT ORGAN CHEM/DK-2100 COPENHAGEN//DENMARK/; RISO NATL LAB,DEPT MAT,POLYMER GRP/DK-4000 ROSKILDE//DENMARK/
Journal: JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, 1993, V115, N15 (JUL 28) , P6477-6481
ISSN: 0002-7863
Language: ENGLISH Document Type: ARTICLE
Abstract: The binding of an eightmer of **peptide nucleic acid** , H-T8-Lys-NH2 (=**PNA** -T8), to a polynucleotide, poly(dA), was studied by flow linear dichroism (LD) and circular dichroism (CD) spectroscopy. Whereas the single **stranded** DNA, due to its high flexibility, does not display any **measurable** LD signal when subjected to shear flow, the complex with **PNA** does. A titration shows that saturation occurs at a stoichiometry of two **PNA** thymine bases per DNA adenine base, indicating the formation of a **triplex** PNA2-DNA complex. The persistence **length** of the adduct remains **small** up to relatively high stoichiometries (above 1:1 T:A) indicating that no significant amounts of **PNA** :DNA duplex are formed. Instead **triplex** stretches seem to form surrounded by flexible parts of single **stranded** poly(dA). Upon approaching the stoichiometry 2:1 of T:A the LD increases dramatically demonstrating that the stiffness of the **PNA** -DNA **triplex** arises from base-base contacts preventing bending of the chain. It is also inferred that the main stiffness of duplex DNA very probably has a similar origin and is not primarily a result of the increased phosphate-phosphate repulsion. Circular dichroism spectra support the conclusion that a **triplex** is formed as the only **PNA** -DNA complex and that it is a right-handed **helix** . The wavelength dependence of the reduced linear dichroism shows that the inclination of the bases from perpendicularity relative to the **helix** axis is **small** . The base conformation of the poly(dA)[**PNA** -T8]2 **triplex** is very similar to that of the conventional poly(dA)[poly(dT)]2 **triplex** .

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4/3,AB/5 (Item 1 from file: 76)
DIALOG(R)File 76:Life Sciences Collection
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01811184 3589237
Right-handed triplex formed between peptide nucleic acid PNA-T sub(8) and poly(dA) shown by linear and circular dichroism spectroscopy
Kim, S.K.; Nielsen, P.E.; Egholm, M.; Buchardt, O.; Berg, R.H.; Norden, B.
Dep. Phys. Chem., Chalmers Univ. Technol., S-412 96 Gothenburg, Sweden
J. AM. CHEM. SOC. vol. 115, no. 15, pp. 6477-6481 (1993)
ISSN: 0002-7863
DOCUMENT TYPE: Journal article LANGUAGE: ENGLISH
SUBFILE: Biochemistry Abstracts 2: Nucleic Acids

duplicate

The binding of an eightmer of **peptide nucleic acid** , H-T sub(8)-Lys-NH sub(2) (=**PNA** -T sub(8)), to a polynucleotide, poly(dA), was studied by flow linear dichroism (LD) and circular dichroism (CD) spectroscopy. Whereas the single **stranded** DNA, due to its high flexibility, does not display any **measurable** LD signal when subjected to shear flow, the complex with **PNA** does. A titration shows that saturation occurs at a stoichiometry of two **PNA** thymine bases per DNA adenine base, indicating the formation of a **triplex** **PNA** sub(2)-DNA complex. The

persistence **length** of the adduct remains **small** up to relatively high stoichiometries (above 1:1 T:A) indicating that no significant amounts of **PNA** :DNA duplex are formed. Instead **triplex** stretches seem to form surrounded by flexible parts of single **stranded** poly(dA). Upon approaching the stoichiometry 2:1 of T:A the LD increases dramatically demonstrating that the stiffness of the **PNA** -DNA **triplex** arises from base-base contacts preventing bending of the chain. It is also inferred that the main stiffness of duplex DNA very probably has a similar origin and is not primarily a result of the increased phosphate-phosphate repulsion. Circular dichroism spectra support the conclusion that a **triplex** is formed as the only **PNA** -DNA complex and that it is a right-handed **helix** . The wavelength dependence of the reduced linear dichroism shows that the inclination of the bases from perpendicularity relative to the **helix** axis i **small** . The base conformation of the poly(dA)[**PNA** -T sub(8)] sub(2) **triplex** is very similar to that of the conventional poly(dA)[poly(dT)] sub(2) **triplex** .

4/3,AB/6 (Item 1 from file: 73)
DIALOG(R)File 98:General Sci Ab/Full-text
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02539444 H.W. WILSON RECORD NUMBER: BGSI93039444
Right-handed triplex formed between peptide nucleic acid **PNA-T8 and poly(dA) shown by linear and circular dichroism spectroscopy.**
Kim, Seog K
Nielsen, Peter E; Egholm, Michael
Journal of the American Chemical Society (J Am Chem Soc) v. 115 (July 28 '93) p. 6477-81
DOCUMENT TYPE: Feature Article
SPECIAL FEATURES: bibl il ISSN: 0002-7863
LANGUAGE: English
COUNTRY OF PUBLICATION: United States

ABSTRACT: The binding of an e[illegible]mer of **peptide nucleic acid** , H-T8-Lys-NH2 (=**PNA** -T8), t[illegible] polynucleotide, poly(dA), was studied by flow linear dichroism (LD) and circular dichroism (CD) spectroscopy. Whereas the single stra[illegible] DNA, due to its high flexibility, does not display any **measurable** LD signal when subjected to shear flow, the complex with **PNA** does. A titration shows that saturation occurs at a stoichiometry of two **PNA** thymine bases per DNA adenine base, indicating the formation of a **triplex** [illegible]2-DNA complex. The persistence **length** of the adduct remains **small** up to relatively high stoichiometries (above 1:1 T:A) indicating that no significant amounts of **PNA** :DNA duplex are formed. Instead **triplex** stretches seem to form surrounded by flexible parts of single **stranded** poly(dA). Upon approaching the stoichiometry 2:1 of T:A the LD increases dramatically demonstrating that the stiffness of the **PNA** -DNA **triplex** arises from base-base contacts preventing bending of the chain. It is also inferred that the main stiffness of duplex DNA very probably has a similar origin and is not primarily a result of the increased phosphate-phosphate repulsion. Circular dichroism spectra support the conclusion that a **triplex** is formed as the only **PNA** -DNA complex and that it is a right-handed helix [illegible] wavelength dependence of the reduced linear dichroism shows that the inclination of the bases from perpendicularity relative to the **helix** axis is **small** . The base conformation of the poly(dA){**PNA** [illegible]2 **triplex** is very similar to that of the conventional poly(dA){poly(dT)}2 **triplex** . Copyright 1993, American Chemical Society.

4/3,AB/7 (Item 1 from file: 99)
DIALOG(R)File 99:Wilson Appl Sci & Tech Abs
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1547094 H.W. WILSON RECORD NUMBER: BAST93046764
Right-handed triplex formed between peptide nucleic acid **PNA-T8 and poly(dA) shown by linear and circular dichroism spectroscopy**
Kim Seog K; Nielsen, Peter E; Egholm, Michael

Journal of the American Chemical Society v. 115 (July 28 '93) p. 6477-81
DOCUMENT TYPE: Feature Article ISSN: 0002-7863

ABSTRACT: The binding of an eightmer of **peptide nucleic acid**, H-T8-Lys-NH2 (=**PNA** -T8), to a polynucleotide, poly(dA), was studied by flow linear dichroism (LD) and circular dichroism (CD) spectroscopy. Whereas the single **stranded** DNA, due to its high flexibility, does not display any **measurable** LD signal when subjected to shear flow, the complex with **PNA** does. A titration shows that saturation occurs at a stoichiometry of two **PNA** thymine bases per DNA adenine base, indicating the formation of a **triplex** PNA2-DNA complex. The persistence **length** of the adduct remains **small** up to relatively high stoichiometries (above 1:1 T:A) indicating that no significant amounts of **PNA** :DNA duplex are formed. Instead **triplex** stretches seem to form surrounded by flexible parts of single **stranded** poly(dA). Upon approaching the stoichiometry 2:1 of T:A the LD increases dramatically demonstrating that the stiffness of the **PNA** -DNA **triplex** arises from base-base contacts preventing bending of the chain. It is also inferred that the main stiffness of duplex DNA very probably has a similar origin and is not primarily a result of the increased phosphate-phosphate repulsion. Circular dichroism spectra support the conclusion that a **triplex** is formed as the only **PNA** -DNA complex and that it is a right-handed **helix**. The wavelength dependence of the reduced linear dichroism shows that the inclination of the bases from perpendicularity relative to the **helix** axis is **small**. The base conformation of the poly(dA){**PNA** -T8}2 **triplex** is very similar to that of the conventional poly(dA){poly(dT)}2 **triplex**.

4/3,AB/8 (Item 1 from file: 144)
DIALOG(R)File 144:PASCAL

11146309 PASCAL No.: 93-0[illegible]
Right-handed triplex formed between peptide nucleic acid PNA-T SUB 8 and poly(dA) shown by NMR and circular dichroism spectroscopy
KIM S K; NIELSEN P E; [illegible]ARDT O; BERG R H; NORDEN B
Charlmers univ. technology, dept. physical chemistry, 412 96 Gothenburg, Sweden
Journal: Journal of the American Chemical Society, 1993, 115 (15) 6477-6481
Language: English
The binding of an eightmer of **peptide nucleic acid**, H-T- SUB 8 -Lys-NH SUB 2 (=**PNA** -T SUB 8), to a polynucleotide, poly(dA), was studied by flow linear dichroism (LD) and circular dichroism (CD) spectroscopy. Whereas the single **stranded** DNA, due to its high flexibility, does not display any **measurable** LD signal when subjected to shear flow, the complex with DNA does. A titration shows that saturation occurs at a stoichiometry of two DNA thymine bases per DNA adenine base, indicating the formation of a **triplex** PNA SUB 2 -DNA complex. The persistence **length** of the adduct remains **small** up to relatively high stoichiometries (above 1:1 T:A) indicating that no significant amounts of **PNA** :DNA duplex are formed

4/3,AB/9 (Item 1 from file: 370)
DIALOG(R)File 370:Science

00500700
Parallel and Antiparallel (G (middle of) GC).inf(2) Triple Helix Fragments in a Crystal Structure
Vlieghe, Dominique; Van Meervelt, Luc; Dautant, Alain; Gallois, Bernard; Precigoux, Gilles; Kennard, Olga
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Science Vol. 273 5282 pp. 1702
Publication Date: 9-20-1996 (960920) Publication Year: 1996
Document Type: Journal ISSN: 0036-8075
Language: English
Section Heading: Reports
Word Count: 2516

Abstract: Nucleic acid triplexes are formed by sequence-specific interactions between single-**stranded** polynucleotides and the double **helix** . These triplexes are implicated in genetic recombination in vivo and have application to areas that include genome analysis and antigene therapy. Despite the importance of the **triple helix** , only limited high-resolution structural information is available. The x-ray crystal structure of the oligonucleotide (GGCCAATTGC) is described; it was designed to contain the d(C-G-C-C) CG.ini(2) fragment and thus provide the basic repeat unit of the **triple helix** . Parameters derived from

17/9/9 (Item 9 from file: 5)
DIALOG(R)File 5:Biosis Previews(R)

10007898 BIOSIS NO.: 199598462816
Symmetry and structure of RNA and DNA triple helices.

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JOURNAL: Biopolymers 36 (3):p333-343 1995
ISSN: 0006-3525
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: Despite wide interest in nucleic acid triple helices, there has been no stereochemically satisfactory structure of an **RNA triple helix** in atomic detail. One triplex structure has previously been proposed based on fiber diffraction and molecular modeling (S. Arnott and P. J. Bond (1973) Nature New Biology, Vol. 244, pp. 99-101; S. Arnott, P. J. Bond, E. Selsing, and P. J. C. Smith (1976) Nucleic Acids Research, Vol. 3, pp. 2459-2470), but it has nonallowed close contacts at every triplet and is therefore not stereochemically acceptable. We propose here a new model for an **RNA triple helix** in which the three chains have identical backbone conformations and are symmetry related. There are no short contacts. The modeling employs a novel geometrical approach using the linked atom least squares (P. J. C. Smith and S. Arnott (1978) Acta Crystallographica, Vol. A34, pp. 3-11) program and is not based on energy minimization. In general, the method leads to a range of possible structures rather than a unique structure. In the present case, however, the constraints resulting from the introduction of a third strand limit the possible structures to a small range of conformation space. This method was used to build a model for **DNA** triple helices (G. Raghunathan, H. Miles, and V. Sasisekharan (1993) Biochemistry, Vol. 32, pp. 1642-1649) subsequently confirmed by fiber-type x-ray diffraction of oligonucleotides (K. Liu, H. T. Miles, K. D. Parris, and V. Sasisekharan (1994) Nature Structural Biology, Vol. 1, pp. 11-12). The above triple helices have Watson-Crick-Hoogsteen (K. Hoogsteen (1963) Acta Crystallographica, Vol. 16, pp. 907-916) pairing of the three bases. The same modeling method was used to investigate the feasibility of three-dimensional structures based on the three possible alternative hydrogen-bonding schemes: Watson-Crick-reverse Hoogsteen, Donohue (J. Donohue (1953) Proceedings of the National Academy of Science USA, Vol. 39, pp. 470-475) (reverse Watson-Crick)-Hoogsteen, and Donohue-reverse Hoogsteen. We found that none of these can occur in either **RNA** or **DNA** helices because they give rise only to structures with prohibitively short contacts between backbone and base atoms in the same chain.

MAJOR CONCEPTS: Biochemistry and Molecular Biophysics
MISCELLANEOUS TERMS: ...KB ...ON ...TION; HYDROGEN BONDING; MOLECULAR MODELING; TRIPLEX ...N PAIRING
CONCEPT CODES:
10062 Biochemical Studies, Nucleic Acids, Purines and Pyrimidines
10506 Biophysics-Molecular Properties and Macromolecules

DNA
nucl./probe/primer
triple/triplex
gene therapy

td015

2

9/4/98
9/4/97

09774826 BIOSIS NO.: 199598229744
Kinetic analysis of triple- helix formation by pyrimidine oligodeoxynucleotides and duplex DNA.

AUTHOR: Xodo Luigi Emilio
AUTHOR ADDRESS: Dep. Biochem. Biophysics Macromol. Chem., Univ. Trieste, Via Giorgieri 1, I-34127 Trieste, Italy

JOURNAL: European Journal of Biochemistry 228 (3):p918-926 1995
ISSN: 0014-2956
DOCUMENT TYPE: Article
RECORD TYPE: Abstract
LANGUAGE: English

ABSTRACT: The kinetics of triplex formation by the pyrimidine oligonucleotide d(CT...C...1, (TFO) and the homopurine cntdot homopyrimidine (R cntdot) ...ple... whose purine strand is d(TGAAAAAGAAAGGAAGAAGAA...GG... (D) was studied using ultraviolet absorbance decay measurements in 30 mM Tris/acetate, pH 6, 50 mM NaCl, 10 mM MgCl-2. The decay curves were obtained by a static method, measuring as a function of time the hypochromicity at 270 nm produced by D and TFO after mixing under conditions favorable for triplex formation. This approach allowed direct measurement of triplex formation as it proceeded. The kinetic experiments were carried out at temperatures below the t-m of the triplex, i.e. at 17 - 33 degree C, and at two different D:TFO ratios, 1:1 and 1:10. When D and TFO were mixed in equimolar amounts, 1.7 mu-M each, the reactions of triplex formation were characterized by half-decay times, t-1/2, of 150-390 s. By contrast, when TFO was in tenfold excess (17 mu-M) cntdot 1-1) over D (1.4 (mu-mol D) cntdot 1-1) the reactions were faster and the t-1/2 decreased to 19-26 s. Different rate equations have been used to describe the kinetics of triplex formation under these two different conditions. Both sets of experiments provided second-order rate constants, k-1, of approximately 10-31 cntdot (mol TFO)-1 cntdot s-1 which showed a slight decrease with temperature. The rate of triplex formation appeared to be about three orders of magnitude lower than the rate of duplex recombination, whose rate constants are in the order of 10-61 cntdot (mol oligomer)-1 cntdot s-1 (Craig, M. E., Crother, D. M. & Doty, P. (1971) J. Mol. Biol. 62, 383-401; Porschke, D. & Eigen, M. (1971) J. Mol. Biol. 62, 361-381; Nelson, J. W. & Tinoco, I. Jr (1982) Biochemistry 21, 5289-5295). The apparent activation energy associated with the rate constants of triplex formation was **small** and negative (E-1 = -26 +- 15 kJ/mol). The first-order rate constants of triplex dissociation, k-1, strongly depended on temperature and were in the range 10-7 s-1 (at 20 degree C) to 10-5 s-1 (at ... C), with an apparent activation energy that was high (... 355 +- 33 kJ/mol). The rate of triplex formation ... showed a significant dependence on the ionic strength (I) of the ... solution. A decrease of I from 130 M to 57 M resulted in a sixfold decrease of the association constant, from 2.16 times 10-3 to 0.36 times ... (mol TFO)-1 cntdot s-1, at 22.5 degree C. The results presented in this study are compared with the kinetic data of triplex ... recently reported in other studies and obtained by different ...
REGISTRY NUMBERS: 254-82-...

DESCRIPTORS:
MAJOR CONCEPTS: Biochemistry and Molecular Biophysics
CHEMICALS & BIOCHEMICALS: HOMOPURINE
MISCELLANEOUS TERMS: ACTIVATION ENERGY; ASSOCIATION CONSTANT; DUPLEX RECOMBINATION; HOMOPURINE; HOMOPYRIMIDINE; STABILITY
CONCEPT CODES:
10062 Biochemical Studies-Nucleic Acids, Purines and Pyrimidines
10506 Biophysics-General Properties and Macromolecules